CERTAIN ASPECTS OF THE ECOLOGY AND BEHAVIOR OF PREPUPAE, PUPAE AND ADULTS OF THE PALE WESTERN CUTWORM, AGROTIS ORTHOGONIA MORR.

by

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THE UNIVERSITY OF ALBERTA

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A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

DEPARTMENT OF ENTOMOLOGY

BY

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EDMONTON, ALBERTA

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ABSTRACT

A description is given of the behavior of Agrotis orthogonia Morr. while forming the earthen cell below ground. A cavity was enlarged by movements of the body and at the same time an oral fluid was exuded which cemented the soil particles to form the cell walls.

At temperatures of 20°, 25° and 30° C. the prepupal period was found to vary directly and the pupal period indirectly with temperature. Variations in the prepupal and the pupal periods were found to be associated with initial prepupal and pupal weights respectively, varying directly with weight. The duration of the prepupal stage was not significantly affected by sex. The pupal stage of the males was slightly longer than that of the females, and this variation was shown to be statistically significant. Saturation deficiency did not affect the duration of the prepupal or pupal stages.

A description is given of eclosion and emergence of the adult. The action of the fore and middle legs ruptured the pupal skin and the moth worked its way out of the exuviae. The soil above the moth was moistened by a fluid exuded from the mouth and spines on the tibiae rasped the softened soil. The moth progressed to the soil surface by moving the loosened soil past and compressing it below the body.

CERTAIN ASPECTS OF THE ECOLOGY AND BEHAVIOR

OF *PERIPATUS*: RUTAS AND ADULTS OF

THE PALE WESTERN CUTWORM,

AGROTIS CRYPTOGONIA MOTH.

ABSTRACT

A description is given of the behavior of *Agrotis cryptogonia* Moth. while forming the earthen cell below ground. A cavity was enlarged by movements of the body and at the same time an oral fluid was exuded which cemented the soil particles to form the cell walls.

At temperatures of 20°, 25° and 30° C. the prepupal period was found to vary directly and the pupal period inversely with temperature. Variations in the prepupal and the pupal periods were found to be associated with initial prepupal and pupal weights respectively, varying directly with weight. The duration of the prepupal stage was not significantly affected by sex. The pupal stage of the males was slightly longer than that of the females, and this variation was shown to be statistically significant. Saturation deficiency did not affect the duration of the prepupal or pupal stages.

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PREFACE

The author wishes to express his thanks and appreciation to the many persons who gave assistance and guidance during the development of this thesis. Chief among these are Dr. C. W. Farstad, Officer in Charge, Field Crop Insect Laboratory, Lethbridge, who, with Mr. L. A. Jacobson and Dr. R. W. Salt, served on a Science Service advisory board and gave generously of their time and contributed many helpful suggestions and criticisms; Dr. B. Hocking, Entomology Department, University of Alberta, and Dr. E. H. Strickland, whose interest and kindly criticisms were gratefully appreciated; Mr. P. Storey and other staff members of the Field Crop Insect Laboratory, Lethbridge, who assisted in rearing the insect material; Mr. C. Reimer, Statistical Research and Service Unit, Ottawa, and Dr. J. E. Andrews, Dominion Experimental Farm, Lethbridge, who assisted in the statistical analysis of the data; Miss B. M. Pehrson, Head Librarian, Science Service, for valuable assistance in obtaining reference material; Messrs. N. E. Kloppenborg and R. Y. Nakamura for the taking and processing of the photographs; Division of Entomology, Science Service, Ottawa, for permission to present the data obtained while in their employ.

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CERTAIN ASPECTS OF THE ECOLOGY AND BEHAVIOR OF PREPUPAE, PUPAE AND ADULTS OF THE PALE WESTERN CUTWORM, AGROTIS ORTHOGONIA MORR.

INTRODUCTION

The pale western cutworm, Agrotis orthogonia Morr. was first described by Morrison in 1876 from specimens collected by G. M. Dodge at Glencoe, Nebraska. The first outbreak, reported by Gibson (1912), occurred in southern Alberta in 1911. Since that date periodic outbreaks have destroyed several hundred thousand acres of cereal crops.

The pale western cutworm occurs in a large portion of the North American Great Plains. Crop losses from this species have occurred throughout the open prairie area of Canada and have extended as far south in the United States as the panhandle of Texas. It is therefore adapted to wide climatic variations.

Much research on the pale western cutworm has been directed towards forecasting outbreaks and towards developing methods of control. These investigations dealt primarily with the larval stage and the oviposition habits of the adults. Field

observations have revealed that unaccountable large reductions in population occur between the last feeding instar and adult emergence. The prepupal and pupal stages have not been studied in detail. Thus little is known about the influences of climatic factors on the duration of these stages.

The investigations reported in this thesis are confined to laboratory studies of the period of the life history from the time larval feeding has stopped to emergence of the adult. This period includes: The formation of the earthen cell by the prepupa, the prepupal and pupal period, eclosion and emergence of the adult. The investigations on the prepupal and pupal stages are directed toward clarifying the influences of temperature and saturation deficiency on the duration of the stages.

Life History

Agrotis orthogonia Morr. has a one year life cycle. The eggs are deposited approximately one quarter inch below the soil surface in the fall, and hatching occurs early the following spring.

The time of hatching varies with latitude as well as with weather conditions prevailing in any given locality. The larvae feed below the ground surface and migrate from plant to plant by burrowing through the soil. A quiescent prepupal period of varying duration appears to have a regulatory function, so that

adult emergence is sufficiently late in the season to avoid fall hatching of the eggs. During the early part of this stage an earthen cell, in which pupation occurs, is formed one and one half to six inches below the soil surface. When the adult emerges it escapes by digging its way to the surface. There does not appear to be a definite pre-oviposition period. The females feed on the nectar of flowering plants and oviposit in dusty soil.

Economic Distribution

Since the first outbreak in 1911, losses due to the pale western cutworm have been reported from prairie areas of Canada. In the United States, Walkden (1950) reported the distribution as confined to the semi-arid and arid sections and stated that outbreaks have occurred in Utah, Montana, Western North Dakota and South Dakota, Wyoming, eastern Colorado, Western Kansas, eastern New Mexico, the Panhandle section of Oklahoma, and north eastern Texas. Only one light infestation has been reported from Nebraska, the state from which the type specimen was collected.

LITERATURE REVIEW

Description and Systematics

A photographic copy of the original description by Morrison (1876) is on the following page.

Agrotis orthogonia nov. sp.

All the tibiæ spinose. Antennæ of the male strongly serrate. Middle of the second joint of palpi black, its outer edge and tip, as well as the third joint, light. Head and thorax gray. Anterior wings dark gray; all the markings well expressed; half-line followed by a white shade line; basal space lighter than the other portions of the wing; interior line forming a very long outward projection below the submedian vein, and another shorter one on the costa, the line is white and distinct, bordered with black on each side, between the submedian and subcostal veins it is straight, except one lobe below the median vein, to which the concolorous, black edged claviform spot is attached; subcostal median and submedian veins white, and contrasting; orbicular spot elliptical, with an outer black ring, within which appears a white annulus, enclosing the gray centre; reniform spot large and of the usual shape, the portion of its black annulus, beneath the median vein, separated and very distinct; exterior line rounded, formed of interspaced luniform marks, followed by a white shade line; subterminal space rather lighter than the median space, terminal space again dark; a series of partially effaced cuneiform marks, before the white subterminal line, which forms two short teeth on the second and third median branches. Posterior wings whitish at the base, with a black terminal band and contrasting white fringes. Beneath whitish, the centre of the median space dark, and the neighborhood of the median vein, on the anterior wings, clothed with long soft hair. Expanse, 34 mm.

Hab. Glencoe, Nebraska. Received from Mr. G. M. Dodge. (No. 66.)

The nearest ally of this fine species is the European *Agrotis vestigialis* Rott.

Morrison (1876) first described the insect as Agrotis orthogonia. Smith (1890) revised the species of the genus Agrotis and placed orthogonia in the genus Porosagrotis. From a single male specimen collected at High River, Smith (1908) described Porosagrotis delorata. Hampson (1908) determined a single specimen from Alberta as Porosagrotis orthogonia. Gibson (1914) reported the first outbreak as being caused by Porosagrotis delorata, since two moths reared from larvae had been identified by Wolley-Dod. Gibson (1914) stated he was convinced P. delorata was Morrison's species orthogonia. The next year Gibson (1915) reported them as P. orthogonia and used the common name "pale western cutworm" for the first time.

Barnes and Benjamin (1926) described as Porosagrotis orthogonia duae a race of orthogonia from California, having more contrasting colors in the wings. They were unable to determine if topotypical orthogonia was delorata. The type of orthogonia was not obtainable, having probably been lost when the Dodge collection was destroyed by fire.

McDunnough (1928) revised the genera of Agrotid moths and considered the genus Porosagrotis as a synonym of Agrotis with delorata and duae as varieties of orthogonia. McDunnough (1938) indicated no change in this classification. The generic name Porosagrotis was used in the literature in Alberta and Montana until 1931.

Prepupal and Pupal Stages

The published literature on the pale western cutworm contains little information regarding construction of the earthen cell. Gibson (1915), in the first report on life history, simply stated that the larvae enter the earth for pupation. Strickland (1916) reported that the mature larvae form small oval cells in the earth. According to Parker, Strand and Seamans (1920), the cells are formed from two to three inches below the soil surface. Eshbaugh (1933) observed that they build pupal cells at a level somewhat lower than where they regularly feed. Walkden (1950) reported that the larvae construct small elliptical cells from three to five inches below the surface, depending on the depth of the plow sole or hardpan. The most detailed description was by Sorenson and Thornley (1941):

> "In entering this quiescent period the larvae burrow from 2 to 6 inches into the soil where each forms an earthen cell. They do not build cocoons but each apparently secretes sufficient saliva with which to form a smooth-walled chamber in the soil. When this moisture dries, the cell wall becomes hard, affording a well insulated, well protected cavity in which the insect passes the remainder of the summer."

Comstock (1920) described the cocoon as an armor for the insect during the vulnerable pupal instar when the insect is unprotected and capable of only limited movement. Most of the literature on cocoon formation deals with those made of silk fibers. Holland (1920) stated that various materials were used

in the construction of pupal cells but in most cases there was an attempt to utilize silk strands to hold the loose material together.

Before 1734 it was believed that earthen cells were no more than an aggregation of soil particles which adhered to the larvae with a glue-like substance secreted through the body wall. Reaumur (1734) pointed out the fallacy of this hypothesis by calling attention to the fact that the cells were larger than the larvae. He described many types of earthen cells, varying from those in which silk fibers were evident in the lining to those which appeared to be made only of compacted earth. A larva which made its cell deep in the earth first had to enlarge the space about it by compressing the earth. The interior surfaces of cells which appeared to be made only of compacted earth were smooth and polished. The cell walls did not rely on the viscosity of damp earth for their strength but on the individual particles being fastened together with silk fibers. The particles were kneaded with the mandibles and moistened with a liquid from the mouth if the soil was too dry. The fibers became visible when a cell was placed in water and stirred so that the soil particles were washed out of the silk matrix. Reaumur mentions trying to make observations through glass but he could only observe the larvae enlarging the cavity; the cell was then constructed over the inside surface of the glass, obstructing his view.

Many Noctuidae, according to Imms (1930) construct earthen cells. They do not appear to utilize silk in the construction of the cells, but rather a fluid secretion which cements the soil particles together.

Strickland (1916) described the construction of the earthen cell by Chorizagrotis auxiliaris:

> "The cell is oval, and, in so far as we have been able to see, it is modelled by the head in conjunction with a revolving motion of the whole body. The internal surface is quite smooth. The fine particles of earth are not held together by silk, though there is evidence of some other salivary secretion which cements them together though it does not make the wall water-proof. The internal measurements of the cell are usually a little under an inch in length and half an inch in diameter. The wall varies in thickness from about one twenty-fourth inch, to one-sixteenth inch when all superfluous loose earth has been removed. The cells may lie in almost any plane in the soil. We have found them most frequently lying more or less horizontally."

Gibson (1915) described the larvae as becoming "full grown", and entering the earth for pupation. The larvae remained in the earth, no change taking place, until pupation was observed. Parker, Strand and Seamans (1921) refer to a period of inactivity before pupation.

> "Although Porosagrotis orthogonia larvae are mature and have practically ceased feeding by the middle of June they do not pupate until nearly a month later. During this period they occasionally feed slightly, but for the most part they remain in a semidormant condition, gradually turning whitish in color and shrinking in size just previous to pupation. This was noticed both in the field and under insectary conditions. Notes taken at Wilsall June 20, 1919, state that on that date cutworms were decreasing in numbers and were nearly all full grown.

"This field was visited again on July 4, when many whitish larvae were found, some of which had formed earthen cells, but no pupae were found in a two-hour search.

"Records kept on 75 larvae in the insectary showed an average period of 20 days of complete inactivity previous to pupation and a period of 26 days in which only very slight feeding took place."

Cook, in 1924 referred to "aestivation" in the soil after the feeding period, in 1926 used the term "prepupal stage", and then in 1930 called it the "prepupal dormant period".

The term "pre-pupal" was defined by Smith (1906) as "that stage in the larva just preceding the change to pupa". Torre-Bueno (1937) defined "prepupal" as "preceding the change to pupa". Neither of these defined the beginning of the stage. Wigglesworth (1950) and Folsom (1934) termed prepupa as an instar. Imms (1930) defined it as a period of quiescence, following construction of the cell, and Comstock (1920), as a period of apparent rest, after the cell was formed, when the wings were outside the pupal cuticula but still covered by the last larval cuticula. Buck (1953) referred to the prepupal period as extending from the time feeding ceased to the time of the last larval moult, and his definition was used in this thesis.

Gibson (1914) reared some larvae sent to him from Alberta by E. H. Strickland and reported that the pale western cutworm had a prolonged prepupal period. He found the larvae entered the soil for pupation on May 28 and the first pupated on June 18.

The pupal stage of these was approximately a month in duration. In 1913 Strickland, at Lethbridge, observed the pupal period varied from 28 to 34 days. The first pupa was found on July 11 and pupation continued till mid-August. Adults emerged in late August and early September.

Similar observations were reported in Montana by Parker, Strand and Seamans (1921). It was noted that the average length of the pupal period in the field and in insectary-reared material was approximately one month. The pupal period of the material reared in the insectary varied from 21 to 40 days, averaging 29½ days. A period of complete inactivity prior to pupation averaged 20 days for the insectary-reared larvae.

According to Seamans and Strickland (1921), the date of pupation depended on how rapidly the larvae had developed.

Crumb (1926) was one of the first workers on cutworms to note that single-brooded species of northern distribution, by prolongation of "a period of retarded development", were able, in more southern latitudes, to fit their life cycle into a full year. The higher average temperature would tend to shorten the time otherwise necessary for completion of the cycle of development, and the prolongation compensates for the shorter time. In the bronzed cutworm Nephelodes emmedonia Cram.,

retardation occurs in both the larval (prepupal) and egg stages.

Cook (1927) stated that some species, such as Euxoa tessellata Harris, and Euxoa idahoensis Grt. hibernate as eggs, pupate immediately when fully fed, and emerge in July. Other species, such as Euxoa pallipennis Sm., Euxoa brevipennis, Sm., and Agrotis orthogonia Morr., interpose a dormant period, two to four weeks in some cases, before pupation.

Cook (1930) found that not only did this prolongation of the prepupal period enable pale western cutworm to extend its range southward, but it also enabled the insect to adjust its life history to different types of season in one locality. In a cool summer the dormant period was short and the pupal period long; thus the moths emerged at the same time as in a hot summer when the dormant period was long. The variations in length of pupal period were not shown in these data.

Eshbaugh (1933) found that at Goodwell, Oklahoma, the prepupal stage seemed to be longer than in Montana, since the moths emerged later. This indicated a longer prepupal period as the range of the insect moved southward and this possibly contributed to its ability to withstand very hot weather during the summer.

Sorenson and Thornley (1940), in Utah, discussing prepupal aestivation, stated that torpidity lasted from one and one

half to two and one half months and that the prepupal period was usually long in comparison with that of other cutworm species. Within field cages in 1936, the pupal period ranged from fifteen to thirty-one days with a mean of twenty-four days, and in 1937 the pupal period ranged from twenty-three to thirty-seven days with a mean of thirty-one days.

Hocking (1953) showed that larval nutrition affected the duration of the prepupal stage of A. orthogonia. At a constant temperature, larvae fed on wheat seedlings had a shorter prepupal period than those fed wheat leaves.

Stanley (1936) compared the length of the prepupal periods of seven species of Noctuids at different levels of constant temperature. He found that higher temperatures accelerated development of all stages of Feltia ducens Wlk. and F. subgothica Haw., except the prepupal stage which was longer. These are single brooded species. The other species, F. annexa Treit., Polia ornithogalli Gn., P. renigera Steph., Caenurgia erechtia Cram., and Laphygma frugiperda A. and S., which are all polyvoltine in Tennessee, had very short prepupal periods which appeared to be shortened by increased temperatures. The increase in length of the prepupal stage of F. ducens and subgothica was interpreted by Stanley as a "natural reaction which allows the species to exist over a wide range of temperature conditions".

Adult Eclosion and Emergence

The literature on pale western cutworm reveals only that the pupae are in earthen cells one and one half to six inches below the soil surface and from these the moths emerge. Cook (1930) did not describe emergence but stated that heavy rains followed by hot weather probably would seal and cake the soil so hard that the adult would not be able to emerge.

Folsom (1934) described eclosion of Danaus archippus. The colors of the imago develop during the last few hours, when they can be seen through the transparent pupal skin. A few convulsive movements of the legs and thorax break the pupal skin in the region of the tongue and legs, a secondary split may occur at the back of the thorax and the butterfly emerges.

Essig (1947) did not describe eclosion but illustrated (after Pfurtscheller) an adult butterfly emerging from the pupal skin. No other reference to Pfurtscheller could be found in the literature.

Many devices for getting out of silken cocoons are discussed in the literature, but there are few references to the adult moth's emergence from an earthen cell several inches below the soil surface. According to Packard (1909), Micropterygidae use activated pupal jaws to break through the cocoon and through the earth above it. Comstock (1920) mentioned the difficult

problem for those insects having sucking mouthparts, and discussed the "cocoon breaker", a toothed crest on the anterior end of the pupa. Holland (1920) described a pupa that wriggled its way to the surface aided by spinous projections on the edge of the somites. These projections prevented backward motion. Imms (1930) stated that Lepidoptera with obtect pupae rarely emerged from the cocoon as pupae. All these authors referred to a fluid which softened or dissolved silk fibers.

Y. Ramachandra Rao (1928) found that Amsacta albistriga (Wlk.) emerged from a depth of five to nine inches in the soil. A sclerotised expansion at the distal end of the fore tibia was terminated by two sharp spines. The exterior spine was small and the interior one sabre-shaped and as long as the tibia. The moth used these spurs as shovels to dig to the surface.

STUDIES ON THE PREPUPAL AND PUPAL STAGES

Materials and Methods

The prepupae and pupae used in this study were reared in the laboratory from eggs deposited by field collected adults. Adults were collected from golden rod (Solidago spp.) and wild sunflower blossoms (Helianthus spp.) along the roadsides near Rosetown, Saskatchewan. The moths feed on the nectar of these

flowers and were easily dislodged into funnel-topped quart sealers. During the warm parts of the day the moths were very active. They remained active until the temperature dropped to 20° C., and from then until the temperature dropped to 15° C. the moths were most easily collected.

The oviposition cages were screen covered glass jars, six inches in diameter by eight inches high, containing one inch of dry soil passed through a 30-mesh screen. Approximately 50 moths were placed in each cage, with a few upright stalks of golden rod bloom for food. Every 24 hours the soil was sifted through the 30-mesh screen to remove the eggs which were then stored (approximately 300 in a 9 by 32 mm. shell vial) at room temperature until the embryos were mature. The eggs were then stored at 0° C..

The larval rearing methods used were modifications of techniques reported by Seamans and McMillan (1935), Jacobson (1952) and Hocking (1953).

The eggs containing mature embryos were placed on moist blotting paper in Petri dishes at 25° C. and the majority of the eggs hatched within 24 hours. Larvae in groups of 25 were reared in 100 by 20 mm. Petri dishes without soil.

Wheat seedlings used for food were grown in the following manner: Thatcher wheat was soaked for two hours in stacking-type

preparation dishes, then drained, covered and placed at 25° C.. Each day the wheat was soaked for a few minutes and drained well. On the fourth day the seedlings were one and one half to two inches high and ready to be used. The coleoptiles and kernels were removed from seedlings fed to first and second instar larvae since the larvae fed in these and were easily lost when the food was changed.

After the third instar, larvae were individually reared in 60 by 15 mm. Petri dishes. Every day the fecal matter and uneaten food were removed from the dishes and fresh food was added. Dates of larval moults were recorded on the covers of the Petri dishes.

Formation of the Pupal Cell

The pupal cell (Fig. 1) is normally constructed by the prepupa below the soil surface. This cell protects the insect during the inactive prepupal and pupal stages. Observations were made to establish how the cell was formed, and whether silk fibers were used in the construction.

When salve tins partly filled with soil were used as rearing containers, it was often observed that some cells were made on the bottom or sides of the container. The cell was not complete, but the surface of the container formed part of the cell wall. This

suggested that if glass containers were used it would be possible to observe the construction of the cell.

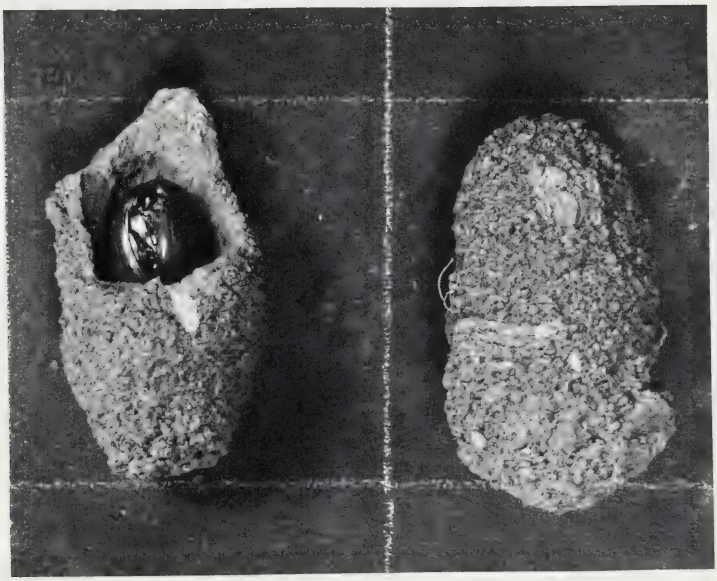


Fig. 1 Pupal cells. The cell on the left is opened to show the enclosed pupa. (1" squares)

Materials and Methods

To restrict larval movements near the glass surface and yet permit the larvae to make their cells at a depth similar to that found in the field, six inch lengths of one and one half inch glass tubing were used as containers. The tubes were stoppered at the bottom and filled to within one inch of the top with

sandy-clay-loam. A rack (Fig. 2) held the tubes at a slight angle from vertical to increase the probability of the cells being constructed against the inside surface of the glass. Petri-dish covers were used as lids.

Fig. 2 Rack holding the tubes for observing formation of the cell.

Individual larvae reared to sixth instar, as previously described, were placed in each container and fed wheat seedlings placed on the soil surface. Uneaten food was removed and fresh food added each day. When it was noted that a larva had not eaten any of the food from the previous day, observations of larval behavior were made whenever the larva was visible through the glass.

Results

After cessation of feeding, the prepupae were often observed moving about in the glass tubes and burrowing downward at a slight angle to the horizontal. Within two days after the cessation of feeding, cell formation commenced at a depth ranging from one and one half inches to the length of the container. Approximately one cell in twelve was made against the inside surface of the glass where it was possible to observe its formation.

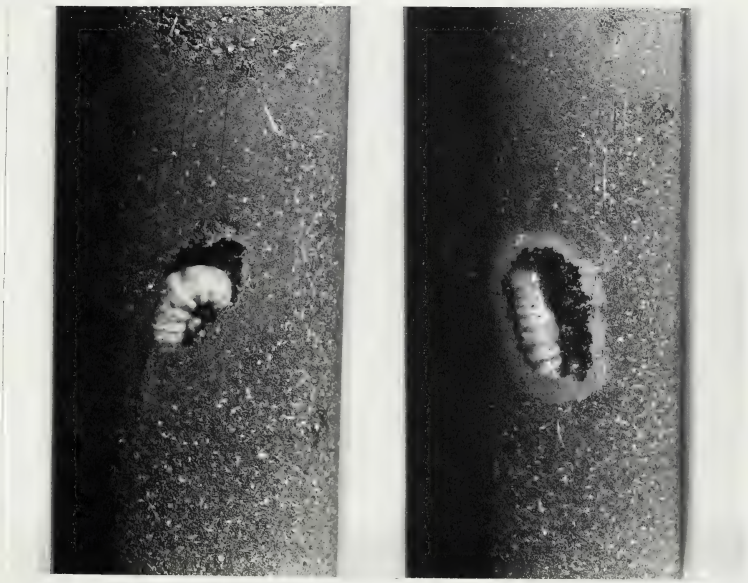


Fig. 3 Prepupa forming cell and completed cell containing pupa. (Actual size)

The prepupa assumed a vertical attitude with the head up: in this position the cavity was slightly enlarged, by rotating the body. The body was moistened with a fluid ejected from the mouth. The main force used to compact the earth appeared to be exerted by the head, with the vertex used as a trowel. While the cell was being enlarged the body was continually rotating, moistening and working the sides of the cavity. The posterior end of the body never left the bottom of the cell, but rather the anterior end bent down and thus enlarged the centre and bottom of the cell. (Fig. 3).

Objects that protruded into the cell, such as a piece of straw, were chewed off. One prepupa enlarged the bottom of its cell by chewing approximately one eighth inch into the cork.

When the size of the cell had been established, the prepupa continued moistening the walls and rotating until the surface was smooth. It then remained inactive, except for a period of approximately two days before pupation. Close observations during and after this activity revealed no attempt by the prepupa to prepare an exit for the adult.

The inside dimensions of cells which were not formed on the glass surface were measured. These cells were also examined for silk fibers. The cells averaged 21 mm. in length and 9 mm. in width, and the pupae from them averaged 16 by 4.5 mm.. The

cell wall thickness in this soil, when all loose earth was removed, averaged 2.5 mm..

Cells were disintegrated in water and examined using a binocular dissecting microscope. No silk fiber could be found.

When dry quartz sand (20-30 mesh) was used as a medium in the tubes the larvae were unable to make cells. A ball of loosely cemented sand approximately one half inch in diameter was found in the tubes and the prepupae came to the surface before pupating or the pupae worked their way to the surface. A similar ball of this sand could not be formed using distilled water.

In dry river sand (85% / 40-60 mesh) cells were formed with walls 10 mm. thick. These walls crumbled very easily. In similar sand kept moist, a cavity was formed but walls were not differentiated.

In dry powdered heavy clay, thin brittle-walled cells were formed. In clay kept moist the cell walls were slightly thicker and more crumbly when dried than those made in powdered clay.

Factors Affecting the Duration of Resting Stages

Materials and Methods

Individual larvae were reared for this experiment at each of three temperatures: 20°, 25° and 30° C.. Every day those larvae which had changed to prepupae were placed in individual glass cells. Those reared at each temperature were then divided at random into three groups and transferred to controlled humidity chambers, with saturation deficits of 3.18, 11.88, and 15.78 mm. of Hg.

The commencement of the prepupal stage is marked by the cessation of feeding and the emptying of the contents of the alimentary canal which causes a drop in weight of approximately 10 per cent. This loss in weight usually occurred within twenty four hours of the last feeding. After moulting to the sixth instar, the individual larvae were weighed daily, and observations were made to determine whether the larva had fed during the previous twenty-four hours. If the larva had fed, the container was cleaned and fresh wheat sprouts added. When it was observed that a larva had not fed and had decreased in weight approximately ten per cent, the individual was considered to have changed to a prepupa. If only a slight loss of weight was recorded, the larva was offered food again and weighed the next day. Commencement of the prepupal period was recorded as

that date on which maximum larval weight was attained and this weight in milligrams was recorded as prepupal weight.

The duration of the prepupal period was determined in days, from the date of maximum weight to the date of change to pupa and the pupal period as the time from change to pupa to emergence. The pupal weight was recorded as the weight at pupation. Sex was determined from antennal characteristics of the moth.

To simulate earthen cells and to allow observations to be made through them, the cells in which individual prepupae were placed were made of 40 mm. lengths of ten mm. bore glass tubing. Pieces of cheesecloth were held in place with rubber bands over each end. To prevent the prepupa from escaping during the first few days, when it would normally be active forming its earthen cell, it was necessary to line the cheesecloth with copper screening. The cheesecloth was required to hold the copper screening in place. When the prepupae became torpid the screening and top covering were removed. The glass cells were placed in the humidity chambers with the head end of the prepupae up.

The desired temperatures were obtained in constant temperature rooms. The variation in the 20° and 25° C. rooms was $\pm$ 0.5° C., as recorded on an eight minute interval electronic strip chart potentiometer actuated by a thermocouple in each room.

The 30° C. room had a variation in temperature of ± 1° C., recorded on a thermograph.

The humidity chambers were glass jars, 10 inches in diameter by 10 inches high. The sheets of glass used as lids were sealed to the ground top edges of the jars with petroleum jelly. A wire rack held the cells in a vertical position. The rack was designed to hold 30 prepupae, in their cells, just inside the chamber walls. This made it possible to observe the insects through the glass without removing them. The cells could be put in or taken out by sliding the lid back just enough to reach in with a pair of forceps, thus only a small fraction of the air in the chamber was disturbed while the cells were being manipulated.

The three saturation deficits used in this experiment were selected as follows: 10 per cent relative humidity was arbitrarily selected as the lowest humidity at 20° C; 50 per cent for the medium relative humidity at 25° C; and 90 per cent as the highest humidity at 30° C.. These relative humidities were then converted to saturation deficiencies. The remaining relative humidities for each temperature were calculated as follows:

$$\frac{\text{relative humidity}}{100} = \frac{\text{saturated vapor pressure} - \text{saturation deficiency}}{\text{saturated vapor pressure}}$$

Solutions of sulphuric acid and distilled water were then mixed, according to Wilson's (1921) tables, to give these desired relative humidities. One litre of solution was used in each chamber. Prior to the experiment the humidities were checked, using an Aminco-Dunmore electric hygrometer, and corrected to within 1 per cent. No change in relative humidity was found during the experiment.

Results

Data obtained for individual insects are given in Appendix A.

The mean number of days duration of the prepupal and the pupal stages for males and females for each of the three humidities at each of the three temperatures are given in Tables I and II, and with sexes grouped, shown graphically in Figure 4.

Since the duration of the prepupal stage appeared to be related to the prepupal weight and the duration of the pupal stage to the weight of pupa, regressions of weight on days duration of stages were calculated. Correlations between the weights and days were also calculated. Calculations are given in Appendix B and C for prepupa and pupa respectively.

An average intra-class regression coefficient for weight of prepupa on days duration of prepupa of 0.027 ± 0.008 was obtained. By the "t" test this was found significant at the one per cent level. ($T = 3.63$, d.f. = 122). This regression coefficient of 0.027 ± 0.008 was interpreted as indicating that an average increment of 10 mgs. in prepupal weight was associated with an average increment of .27 days or approximately one quarter day in the duration of the prepupal stage.

A correlation coefficient of $+0.3123$ between prepupal weight and days prepupa was obtained. Interpreted as r^2 this

indicated that 9.7 per cent of the variation in number of days prepupal period was associated with variation in prepupal weight.

The average intra-class regression coefficient for weight of pupa on days in the pupal stage was found to be 0.012 ± 0.004, which is significant at the one per cent level ($T = 3.16$, d.f. = 122). This indicated that an average increment of 10 mgs. in pupal weight was associated with an average increment of .12 days or approximately 1/8 day in the duration of the pupal stage.

The correlation coefficient between pupal weight and days pupal stage was + 0.276. When this was interpreted as r^2, it indicated that .076 or 7.6 per cent of the variation in the duration of the pupal stage was associated with variation in pupal weight.

An analysis of variance was used to test the effect of temperature, humidity and sex on the duration of the prepupal and the pupal stages. Since treatment means and variances tended to be related, individual measurements (Appendix A) were converted to logarithms.

The analysis of variance with disproportionate numbers in the different classes and with three factors involved is very cumbersome. However the ratio of males to females varied only slightly from one class to another and the humidity effects did not appear to be appreciable (TablesI and II). Therefore it was assumed the effect of humidity was negligible and the analysis for

TABLE I

Mean Number of Days' Duration of the Prepupal Stage of Agrotis orthogonia Morr.

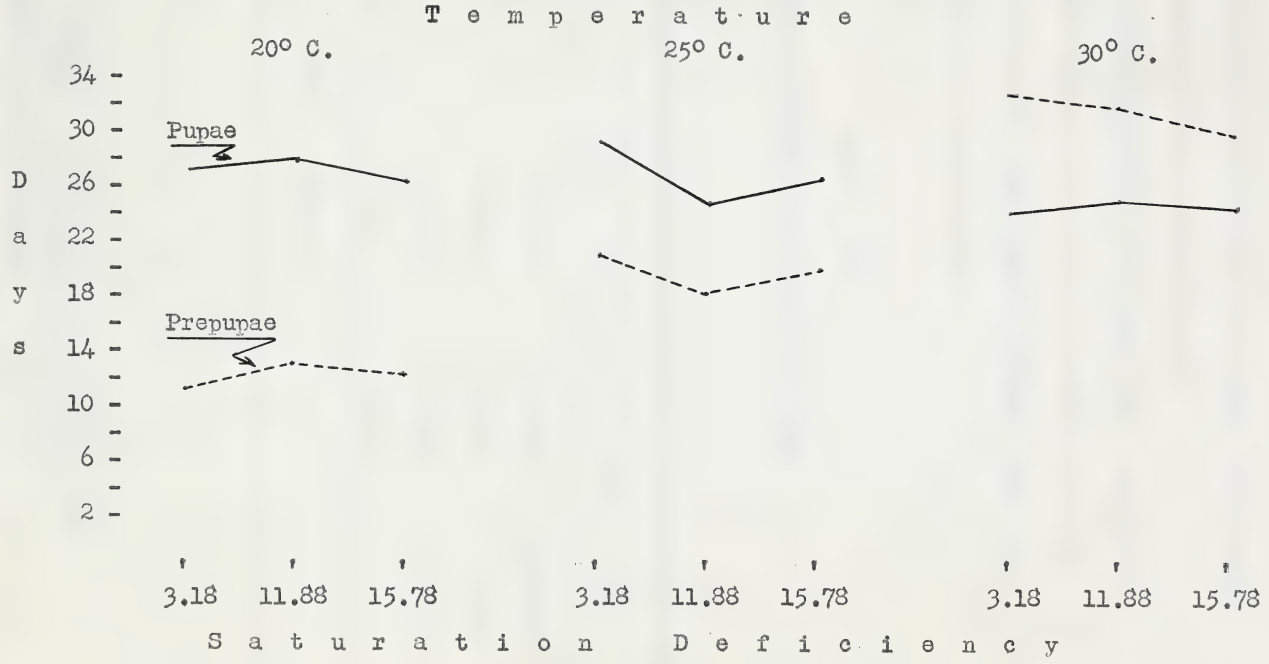
Saturation deficiency (mm. of Hg.)	Temperature 20° C. Males	20° C. Females	20° C. Mean ♂+♀	25° C. Males	25° C. Females	25° C. Mean ♂+♀	30° C. Males	30° C. Females	30° C. Mean ♂+♀
3.18	11.5	12.5	11.8	22.1	19.3	20.9	32.3	33.6	32.9
11.88	13.4	13.0	13.2	14.8	20.2	18.3	31.6	31.9	31.7
15.78	13.0	12.2	12.4	14.6	23.8	20.2	30.5	30.3	30.3
Mean	12.3	12.4	12.4	17.6	21.4	19.9	31.8	31.7	31.8

TABLE II

Mean Number of Days' Duration of the Pupal Stage of Agrotis orthogonia Morr.

Saturation deficiency (mm. of Hg.)	Temperature								
	20° C.			25° C.			30° C.		
	Males	Females	Mean ♂+♀	Males	Females	Mean ♂+♀	Males	Females	Mean ♂+♀
3.18	27.6	27.2	27.5	30.7	28.0	29.5	24.6	24.1	24.4
11.88	28.0	28.0	28.0	26.0	25.3	25.6	25.2	25.0	25.1
15.78	27.4	27.2	27.2	27.1	26.9	27.0	24.0	24.5	24.5
Mean	27.7	27.3	27.5	28.4	26.7	27.4	24.9	24.6	24.7

FIGURE 4 Duration of the Prepupal and Pupal Stages



a two way classification of data with proportional sub-classes as described by Snedecor (1946) could be used.[1]

The procedure of analysis of these data, using the logarithmic transformations, are given in Appendix D and E. The analysis of variance for days prepupa and days pupa are given in Tables III and IV respectively.

TABLE III

Analysis of Variance for Days as Prepupa

Source of Variance	d.f.	Sums of Squares	Mean Square	F
Temperature	2	3.9750	1.9875	148.8764**
Sex	1	0.0299	0.0299	2.2397
Temp. x Sex	2	0.0322	0.0161	1.2059
Within Sub-classes	135	1.8029	0.01335	
Total	140	7.7447		

1. Suggested in personal communication from C. Reimer, Statistical Research and Service Unit, Ottawa.

TABLE IV

Analysis of Variance for Days as Pupa

Source of Variance	d.f.	Sums of Squares	Mean Squares	F
Temperature	2	.0655	.0328	65.6**
Sex	1	.0059	.0059	11.8**
Temp. x Sex	2	.0022	.0011	2.2
Within Sub-classes	135	.0736	.0005	
Total	140	.1472		

Discussion

These investigations on the prepupal stage showed that, of the factors measured, only temperature and weight affected the duration of the stage. Sex and humidity appeared to have little effect on the duration. The experimental design did not limit the temperature effect to the prepupal stage. The larvae were reared at the temperature at which the length of the prepupal stage was measured and some differences may be attributed to this pre-treatment.

The only measurable variable in pre-treatment was temperature. The eggs were from the same source, larvae were distributed to the three temperatures at random and fed similarly. The average prepupal weight (maximum larval weight) for larvae reared at 30° C. was only 3 mgs. greater than the average weight at 20° C. (Appendix A). It was found that an average increase of 10 mgs. was associated with an average increase of .27 days, and thus an increase of 37 mgs. would be required to increase the duration one day. Therefore larval nutrition as reflected in prepupal weight had little effect on the differences between temperatures. Prior investigations on larval development (Blakeley, Jacobson and Lindsay, 1952) in which the same three temperatures and similar food were used indicated that the larval stage was significantly shorter at 30° C. than at 20° C.. These data were not recorded for this study, but duration of larval stage may be a factor.

The adaptation whereby at higher temperatures the prepupal period is extended has not been referred to in the literature on pale western cutworm as a form of diapause. Diapause, according to Andrewartha (1952) is an adaptation which enables insects to persist in regions otherwise unfavorable for permanent habitation or which induces a rhythm in the life cycle synchronized with the environmental rhythm.

Wheeler (1893) used the word "diapause" as an embryological term, to describe a more or less stationary stage in the morphogenesis of the embryo of Xiphidium. Henneguy (1903) generalized the term to include any physiological state of rest at any stage of development. Shelford (1929) further defined "diapause" as a dormancy which is more or less spontaneous as opposed to "quiescence" where development is temporarily inhibited by unfavorable environmental conditions and will resume immediately when returned to a favorable environment.

Andrewartha (1952) defines "diapause stage" as "that stage in the life cycle during which morphogenesis is more or less at a standstill;" and "diapause development" as "the physiological development, or physiogenesis which goes on during the diapause stage in preparation for the active resumption of morphogenesis."

The condition during the prepupal stage of the pale western cutworm fits these definitions since the term "more or less at a standstill" is used. If the temperature range required for "diapause development" includes the temperature at which the insect is being reared, then "diapause development" will proceed. If the rearing temperature does not fall within the temperature range for diapause development then physiogenesis will not proceed unless and until the insect is exposed to temperatures within this range.

In the pale western cutworm the temperature range for larval development includes all three temperatures, 20°, 25°, and 30° C., used in this experiment. The optimum temperature for larval development is above 25° C.. The temperature range for prepupal development also includes all three temperatures with an optimum temperature below 25° C. since according to the results at 20, 25 and 30° C., the mean number of days duration of the prepupal stage were 12.3, 19.9, and 31.8 respectively. At these temperatures development of both stages will proceed, and there is therefore no arrest in development between stages. Considering the prepupal stage as a "diapause stage", morphogenesis is not inhibited altogether at any one of the three temperatures used in this experiment. The rate of "diapause development" is merely slowed down as the temperature is increased from the optimum for this development.

STUDIES ON ADULT ECLOSION AND EMERGENCE

Adult Eclosion

Fifty pupae reared at 25° C. were used to observe eclosion. The individual pupae were kept in 60 by 15 mm. Petri dishes. The date of pupation was recorded on the cover and the dishes were arranged in a single layer in trays, according to the date.

Since eclosion had never been observed by the author in earlier studies, it was felt that the entire process was very rapid and would therefore require intensive observation. In the study on the duration of the pupal period at 25° C. it was found that eclosion took place in 56 per cent of the pupae between 27 and 29 days after pupation. Therefore after the twenty-seventh day the pupae were observed through a binocular dissecting microscope as continuously as possible.

Pupal exuviae were examined under the dissecting microscope to determine if there was any variation in the opening resulting from eclosion.

Eclosion was only witnessed in two of the fifty pupae under observation, and in both cases the process was completed in less than five minutes. The adult became very active within the pupal case. With a forward and downward movement of the fore and middle legs the pupal case began to split. The spines on the tibiae of the fore legs (Fig. 5) appeared to grip the cuticle and prevent the legs from slipping. Separation from the pupal skin first occurred between the prothorax and vertex, and continued down the sutures between the mesothoracic wings and the antennae. The flap thus formed remained hinged at the distal end of the antennae and proboscis (Fig. 6). With the head and thorax out of the exuviae, the abdomen expanded and contracted until it was

withdrawn and eclosion was completed. The moth was quite moist, probably with a moulting fluid used to weaken the cuticle.

Fig. 5 Fully developed adult within pupal skin, showing position of fore and mid legs before eclosion with spines on fore tibiae gripping the cuticle. (x16)

The exuviae of all 50 pupae were examined. No variation in the opening (Fig. 6) was found.

Fig. 6 Fully developed pupa (left) and pupal exuviae showing typical opening (right) (x3½)

Emergence through the Soil

The glass tubes in which earthen cells were formed on the inside surface of the glass were used in the study of adult emergence. These were supplemented with similar tubes filled with sandy clay loam. The soil was compressed into the tube and the cork removed from the bottom. Artificial earthen cells were made at the bottom of the tube on the inside surface of the

glass, using a glass stirring rod dipped in water. These cells were made according to the measurements found in the study of cell formation.

Pupae were placed in the cells, anterior end first, and the corks replaced. The tubes were then placed in a rack and tilted so that the emerging moths could be observed through the glass as they worked up through the soil. Date of pupation and expected date of emergence were recorded on the tube.

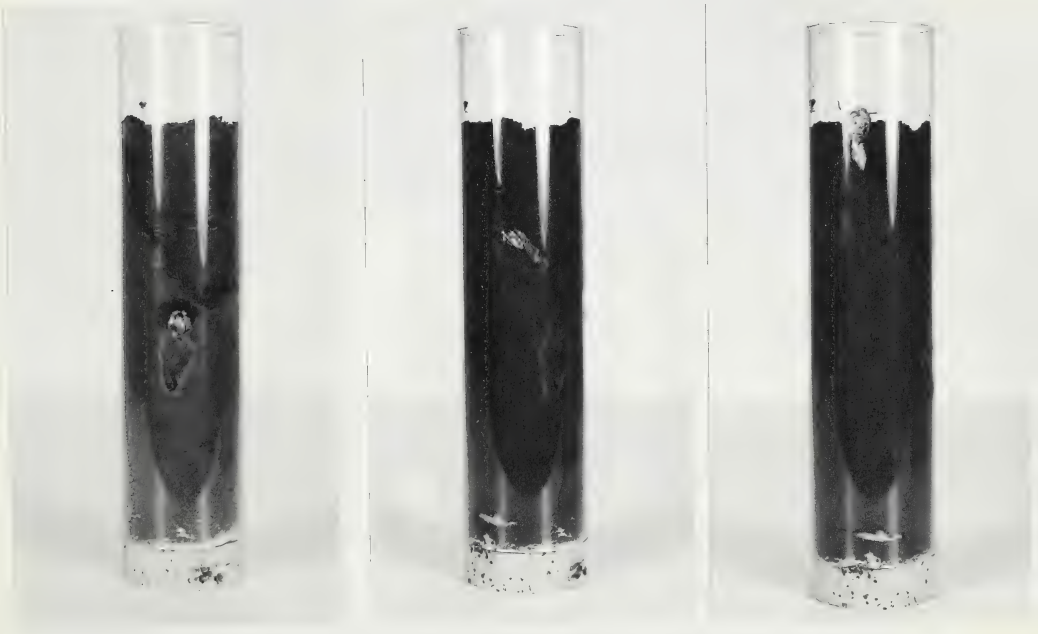
Tubes of corrugated cardboard 18 inches in height and six inches in diameter lined with wax paper were used to determine the distance a moth could move through the soil. The following soils, both moist and dry, were used: sand, sandy clay loam, and heavy clay.

One gallon crocks were filled with the various soils and seven artificial cells were made into the surface of each. Individual pupae were placed in each of these cells, anterior end up. The cells were then completed with a slightly moistened pad of soil. The tubes, described above, were then placed over these and filled with the various soils. Water was added occasionally to the soil in the moist series.

One week after the calculated date of emergence, the number of moths on the surface was recorded for each treatment. Soil was then removed in one inch layers, cutting the

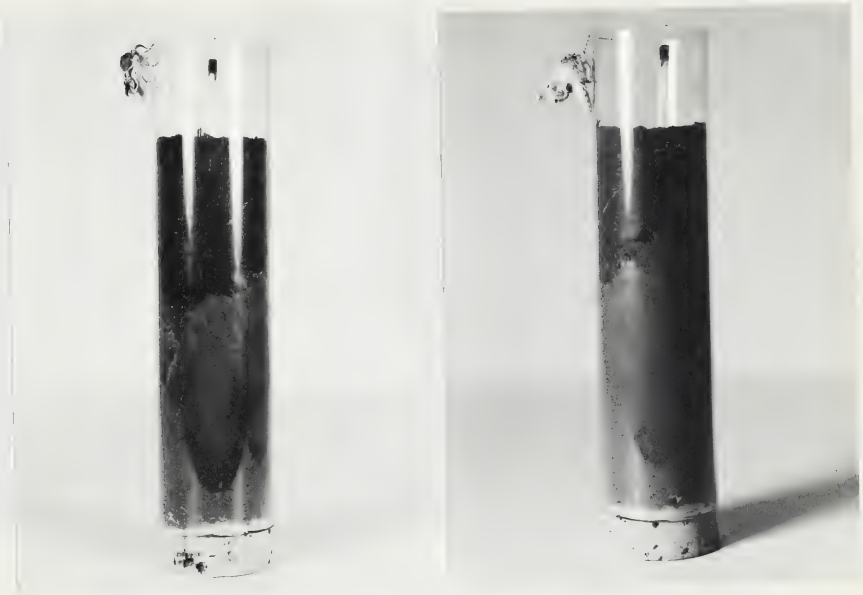
tubes when necessary. Each layer was sifted, and the distance the moths travelled was recorded.

Several moths were observed in the process of digging their way to the surface. Complete emergence from the earthen cell to the elongation of the wings was only observed in one tube (Figs. 7 and 8). The moth climbed to the top of the cell,



A. B. C.

Fig. 7 Moth in process of digging its way to surface.



A. B.

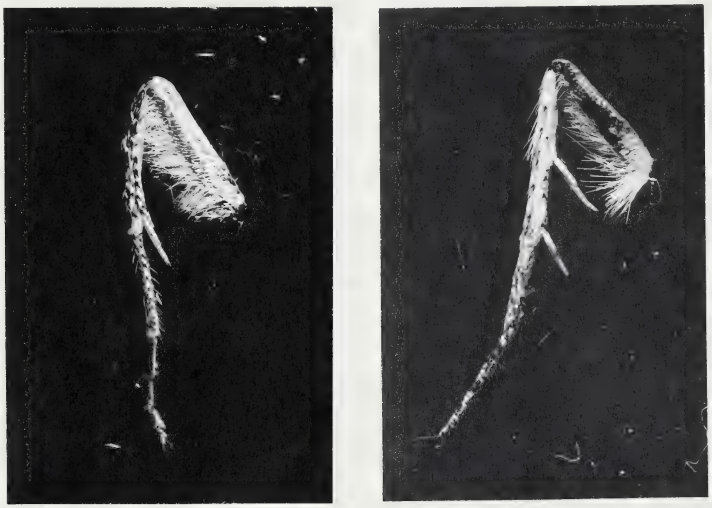
Fig. 8 Moth before and after elongation of the wings.

then braced itself with its mid and hind legs. Droplets of fluid then exuded from the mouth. The uncoiled proboscis appeared to be pressed against the wall of the cell to open the mouth at the apex of the proboscis. The fluid was then placed on the roof of the cell until the soil was quite moist. Heavily sclerotised spines (Figs. 5 and 9) along the margins of the fore tibiae were used as scrapers to rasp away the moistened earth (Fig. 10). The same digging action was used to complete the tunnel to the soil

Fig. 9 Fore leg showing heavily sclerotised spines on tibia. (x17)

Fig. 10 Moth in process of digging through the soil. (x6½)

surface. Fluid was exuded from the mouth whenever necessary to soften the earth. As the soil was removed, it was moved down past the body with the middle and hind legs. The spines on these legs (Fig. 11) were used to hold the earth as it was



A. B.

Fig. 11 Middle (A) and hind leg (B) showing spines used to move the earth downward. (x 7)

moved downward. The tip of the abdomen was in continual motion, compacting the soil beneath the moth, as it progressed upward. The moth rotated while digging so as to produce a round tunnel.

To determine whether the upward direction of movement was the result of the original orientation of the pupa or was negative geotaxis, the tube was slowly turned on its side, then

inverted. The moth changed its direction of tunneling and continued to work upward as the tube was turned, which indicated that the action was negative geotaxis.

Figure 7-B shows the moth in almost a horizontal position, which resulted when the dry soil above the moth was removed and substituted with moist compacted soil. This was done to retard the moth and give more time to photograph the procedure. The moth took the line of least resistance rather than tunnel through the compacted soil.

Eleven moths emerged through 18 inches of dry soil in the cardboard tubes, four from the sand, four from the sandy clay loam, and three from the heavy clay. One moth was found dead at the 14-inch level in the dry sand. The remaining eight pupae were examined, and were found to contain fully developed adults that were dead. No moths emerged in the moist series. When the pupae were examined the majority in each soil contained dead adults; the remainder had decomposed.

Discussion

Where eclosion had not taken place it appeared that the artificial earthen cells had disintegrated and that the soil, being in contact with the pupae, probably prevented the moth from forcing its way out of the pupal skin. It was not possible to determine whether the cells of those that emerged were complete

at the time of eclosion. It appears that the additional space provided by the earthen cell is required for eclosion. Without this additional space, unless the earth is compacted as firmly behind the moth as that through which it is tunneling, the moth would not be able to emerge.

SUMMARY AND CONCLUSIONS

The investigations described in this thesis were conducted at the Field Crop Insect Section, Science Service Laboratories, Lethbridge. The prepupae and pupae of Agrotis orthogonia Morr. used in this study were reared from eggs deposited by field collected adults.

The pupal cells are normally formed below the soil surface. Glass walled containers filled with soil were used in this study to observe construction of the cells. Observations were made on cells constructed against the inside surface of the glass. Movements of the body enlarged the cavity in the soil. A fluid, exuded from the mouth, cemented the soil particles to form the cell walls. Observations on cell formation in various soil types are also described.

Data are presented on the effect of temperature, saturation deficiency, weight and sex on the duration of the prepupal and pupal period. Temperatures of 20°, 25° and 30° C. were used

for this experiment. The prepupal period was found to vary directly and the pupal period indirectly with temperature. The variation in the prepupal period was from 12.4 days at 20° C. to 31.8 days at 30° C.. The variation in the pupal period was from 27.4 days at 20° C. to 24.7 days at 30° C..

These results indicate that a diapause exists during the prepupal stage, which does not inhibit morphogenesis altogether but merely slows down the rate of development at the higher temperatures.

The differences in duration of the prepupal stage may be affected by the temperature during the larval feeding stage. Further investigations should be undertaken to determine the effect of temperature during the larval feeding period on the duration of the prepupal stage.

An average increment of 10 mgs. in initial prepupal weight was associated with an average increment of approximately one quarter day in the duration of the prepupal stage. An average increment of 10 mgs. in initial pupal weight was associated with approximately one eighth day duration of the pupal stage.

The duration of the prepupal stage was not significantly affected by sex. The pupal stage of the males was slightly longer than that of the females, and this variation was shown to be statistically significant.

Saturation deficiencies of 3.18, 11.88, 15.78 mm. of Hg. did not significantly affect the duration of the prepupal and pupal stages.

Observations showed that eclosion of the adult was accomplished by the front and middle legs exerting an outward and downward pressure which ruptured the pupal skin along definite sutures. It appeared that a moulting fluid had first softened the cuticle. Eclosion then took place through the opening thus formed. No variation in this opening was found.

Emergence of the adult was observed through glass walled containers. The soil above the moth was moistened with a fluid exuded from the mouth. This softened soil was then rasped with heavily sclerotised spines on the tibiae of the fore legs. The loosened soil was moved past the body and compressed below until the moth reached the surface.

Experimental evidence indicates that the space provided by the earthen cell is necessary for emergence. The prepupae were unable to construct cells in pure quartz sand or in moist river sand and very crumbly cells were formed in dry river sand and moist clay. These findings may be of value in the field to interpret decreases in populations during the prepupal and pupal periods. Without the protection of the hard-walled earthen cells the prepupae and pupae could be more susceptible to predators.

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Appendix A: Data obtained from observations on individual Agrotis orthogonia Morr. at three levels of saturation deficiency at 20°, 25° and 30° C.

20° C.						25° C.						30° C.					
S.D. (mm. Hg.)	Sex	Weight (mgs.) Prepupa	Pupa	Duration (days) Prepupa	Pupa	S.D. (mm. Hg.)	Sex	Weight (mgs.) Prepupa	Pupa	Duration (days) Prepupa	Pupa	S.D. (mm. Hg.)	Sex	Weight (mgs.) Prepupa	Pupa	Duration (days) Prepupa	Pupa
3.18	♂♂	446	260	8	26	3.18	♂♂	712	340	29	28	3.18	♂♂	740	419	26	26
		645	295	11	26			718	396	37	29			512	293	36	25
		623	283	12	26			1194	704	37	30			534	293	38	24
		614	317	13	28			572	332	21	46			592	245	27	23
		497	296	7	24			770	439	25	31			570	321	24	26
		585	339	8	28			708	421	22	29			622	329	36	24
		622	347	13	28			574	324	7	26			622	387	45	26
		654	353	13	29			622	356	9	28			736	384	26	23
		706	389	15	31			706	409	12	29						
		548	312	14	27								♀♀	676	357	46	25
		768	319	12	31		♀♀	622	308	17	28			664	363	27	23
								794	404	33	28			674	341	34	22
	♀♀	694	345	11	26			658	334	26	28			680	338	34	27
		639	324	11	24			698	393	8	28			656	326	50	19
		621	383	16	28			816	479	29	30			780	414	16	26
		756	438	13	29			564	310	11	25			766	414	44	25
		676	414	14	28			592	373	11	29			644	318	18	26
		686	356	10	28												
						11.88	♂♂	640	391	15	28	11.88	♂♂	644	294	24	25
11.88	♂♂	655	359	10	27			416	348	17	27			520	291	42	25
		719	393	12	29			806	409	16	23			620	317	31	25
		584	335	14	29			702	407	16	28			570	299	38	27
		656	348	15	28			562	325	10	24			534	310	30	24
		696	349	16	27									650	357	35	26
							♀♀	734	372	12	29			662	324	23	26
	♀♀	546	263	9	25			760	401	26	25			638	318	37	26
		636	402	9	29			700	304	36	28			750	356	29	24
		741	388	12	29			644	351	12	29			600	333	26	25
		711	390	16	28			854	429	47	21			634	281	33	24
		692	404	19	29			640	372	9	23						
								764	438	11	27		♀♀	622	338	31	21
15.78	♂♂	574	304	9	27			558	292	11	22			738	284	41	24
		600	351	14	29			464	220	18	24			620	329	27	27
		576	341	17	27									664	342	41	24
		672	371	14	28	15.78	♂♂	718	365	13	28			670	315	27	24
		680	381	11	26			636	319	27	27			682	385	33	28
								750	383	14	30			706	376	22	29
	♀♀	664	294	12	27			712	389	25	29			714	381	33	23
		799	419	11	28			608	256	7	24						
		638	344	9	26			578	318	7	28	15.78	♂♂	530	287	29	24
		429	214	9	24			570	313	9	24			660	319	32	24
		609	302	11	26												
		768	460	11	28		♀♀	648	316	38	26		♀♀	672	358	27	24
		534	302	9	28			754	353	25	28			694	358	23	25
		589	326	12	27			542	312	9	24			646	335	42	22
		694	406	20	27			712	364	28	26			656	330	40	25
		782	428	16	29			592	342	22	27			710	370	39	24
		748	400	14	30			606	271	40	24			688	330	30	22
		824	436	14	27			696	413	32	29			700	352	30	29
		688	304	10	26			706	357	31	27			664	323	22	24
								766	432	19	30			550	325	19	25
								570	346	7	27			722	327	31	26
								744	431	11	28			740	356	30	24
	Mean	651	352	12.4	27.5			681	368	19.9	27.4			654	336	31.8	24.7

Appendix B: Regression of 'days prepupa' (Y) on 'weight prepupa, mgs.' (X) within treatment, within sex

TABLE 1a

Computation of Sums of Squares and Products for 20° C. Data in Table 2

Class	20°,3.2,F	20°,3.2,M	20°,11.9,F	20°,11.9,M	20°,15.8, F	20°,15.8,M
n	6	11	5	5	13	5
ΣX	4072	6708	3326	3310	8766	3102
ΣX^2	2774706	4172464	2236078	2201794	6068148	1935236
$(\Sigma X)^2/n$	2763531	4099660	2212455	2191220	5910981	1924481
Σx^2	11175	81804	23623	10574	157167	10755
ΣXY	50751	78346	44054	44330	108661.0	40246.0
$(\Sigma X)(\Sigma Y)/n$	50900.0	76837.1	43238.0	44354.0	106540.6	40326.0
Σxy	-149.0	1508.9	816.0	-24.0	2120.4	-80.0
ΣY	75	126	65	67	158	65
ΣY^2	963.0	1514	923	921	2042	883
$(\Sigma Y)^2/n$	937.5	1443.3	845.0	897.8	1920.3	845.0
Σy^2	25.5	70.7	78.0	23.2	121.7	38.0
$(\Sigma xy)^2/\Sigma x^2$	2.0	27.8	28.2	.1	28.6	.6
$\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	23.5	42.9	49.8	23.1	93.1	37.4

Appendix B: Regression of 'days prepupa' (Y) on 'weight prepupa, mgs.' (X) within treatment, within sex

TABLE 1b

Computation of Sums of Squares and Products for 25° C. Data in Table 2

Class	25°,3.2,F	25°,3.2,M	25°,11.9,F	25°,11.9,M	25°,15.8,F	25°,15.8,M
n	7	9	9	5	11	7
ΣX	4744	6576	6118	3324	7336	4572
ΣX^2	3271904	5084248	4270364	2244880	4954872	3018112
$(\Sigma X)^2/n$	3215077	4804864	4158880	2209795	4892445	2986169
Σx^2	56827	279384	111484	35085	62427	31943
ΣXY	95848.0	156318.0	130288.0	49786.0	176438.0	68238.0
$(\Sigma X)(\Sigma Y)/n$	91491.4	145402.7	123719.6	49195.2	174730.2	66620.6
Σxy	4356.6	10915.3	6568.4	590.8	1707.8	1617.4
ΣY	135	199	182	74	262	102
ΣY^2	3201.0	5403.0	5116.0	1126.0	7534.0	1898.0
$(\Sigma Y)^2/n$	2603.6	4400.1	3680.4	1095.2	6240.4	1486.3
Σy^2	597.4	1002.9	1435.6	30.8	1293.6	411.7
$(\Sigma xy)^2/\Sigma x^2$	334.0	426.5	387.0	9.9	46.7	81.9
$\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	263.4	576.4	1048.6	20.9	1246.9	329.8

Appendix B: Regression of 'days prepupa' (Y) on 'weight prepupa, mgs.' (X) within treatment, within sex

TABLE 1c

Computation of Sums of Squares and Products for 30° C. Data in Table 2

Class	30°,3.2,F	30°,3.2,M	30°,11.9,F	30°,11.9,M	30°,15.8,F	30°,15.8,M
n	8	8	8	11	11	2
$\leq X$	5540	4968	5416	6822	7442	1190
$\leq X^2$	3854776	3137088	3679080	4271836	5060596	716500
$(\leq X)^2/n$	3836450	3085128	3666632	4230880	5034851	708050
$\leq x^2$	18326	51960	12448	40956	25745	8450
$\leq XY$	185636.0	158946.0	173194.0	214050.0	226448.0	36490.0
$(\leq X)(\leq Y)/n$	186282.5	160218.0	172635.0	215823.3	225289.6	36295.0
$\leq xy$	-646.5	-1272.0	559.0	-1773.3	1158.4	195.0
$\leq Y$	269	258	255	348	333	61
$\leq Y^2$	10173.0	8718.0	8443.0	11374.0	10649.0	1865.0
$(\leq Y)^2/n$	9045.1	8320.5	8128.1	11009.5	10080.8	1860.5
$\leq y^2$	1127.9	397.5	314.9	364.5	568.2	4.5
$(\leq xy)^2/\leq x^2$	22.8	31.1	25.1	76.8	52.1	4.5
$\leq y^2 - (\leq xy)^2/\leq x^2$	1105.1	366.4	289.8	287.7	516.1	--

Appendix B:

TABLE 2

Regression Data in 18 Classes of Prepupa

Class				Sums of squares and products			Errors of estimate	
Temp.	S.D.	Sex	d.f.	$\leq x^2$	$\leq xy$	$\leq y^2$	SS	d.f.
20° C.	3.2	F	5	11175	-149.0	25.5	23.5	4
		M	10	81804	1508.9	70.7	42.9	9
	11.9	F	4	23623	816.0	78.0	49.8	3
		M	4	10574	-24.0	23.2	23.1	3
	15.8	F	12	157167	2120.4	121.7	93.1	11
		M	4	10755	-80.0	28.6	37.4	3
25° C.	3.2	F	6	56827	4356.6	597.4	263.4	5
		M	8	279384	10915.3	1002.9	576.4	7
	11.9	F	8	111484	6568.4	1435.6	1048.6	7
		M	4	35085	590.8	30.8	20.9	3
	15.8	F	10	62427	1707.8	1293.6	1246.9	9
		M	6	31943	1617.4	411.7	329.8	5
30° C.	3.2	F	7	18326	-646.5	1127.9	1105.1	6
		M	7	51960	-1272.0	397.5	366.4	6
	11.9	F	7	12448	559.0	314.9	289.8	6
		M	10	40956	-1773.3	364.5	287.7	9
	15.8	F	10	25745	1158.4	568.2	516.1	9
		M	1	8450	195.0	4.5	--	--
	Sums		123	1030133	28169.2	7897.2	6320.9	105

Appendix B:

TABLE 3

Analysis of Errors of Estimate from Average Regression Within Groups

Source of variation	d.f.	SS	MS
Deviations from average intra-class regression, $7897.2 - \frac{(28169.2)^2}{1030133}$	122	7126.9	
Deviations from individual intra-class regressions	105	6320.9	60.20
Differences among intra-class regressions	17	806.0	47.41

Since the 18 individual regression coefficients do not differ significantly (Table 3) they were pooled to give an average intra-class regression coefficient, b_a , viz.,

$$b_a = \Sigma\Sigma xy / \Sigma\Sigma x^2 = \frac{28169.2}{1030133} = 0.02735$$

Its standard error, s_b, is calculated from the mean square of deviations from average regression, i.e.,

$$s_b = \sqrt{\frac{7126.9/122}{x^2}} = \sqrt{\frac{58.42}{1030133}}$$

$$\sqrt{.00005671} = 0.00753$$

To test b_a against zero,

$$t = b_a / s_b = .02735/.00753 = 3.63$$

which, with 122 d.f., is significant at the 1% level.

Thus the average intra-class regression coefficient was 0.027 ± 0.008 or that an average increment of 10 mg. in prepupal weight was associated with an average increment of approximately ¼ day in prepupal period.

Alternatively, the observed relationship between 'prepupal weight' and 'prepupal days' can be expressed with reference to the intra-class correlation coefficient.

$$r = \frac{\Sigma\Sigma xy}{\sqrt{\Sigma\Sigma x^2 \Sigma\Sigma y^2}} = \frac{28169.2}{\sqrt{8135166328}}$$

$$\frac{28169.2}{90195} = .3123$$

$$r^2 = .097$$

Therefore approximately 10% of the intra-class variation of the variable prepupal days was associated with prepupal weight.

Appendix C: Regression of 'days pupa' (Y) on 'weight pupa, mgs.' (X) within treatment, within sex

TABLE 1a

Computation of Sums of Squares and Products for 20° C. Data in Table 2

Class	20°,3.18,F	20°,3.18,M	20°,11.88,F	20°,11.88,M	20°,15.78,F	20°,15.78,M
n	6	11	5	5	13	5
ΣX	2260	3582	1847	1784	4635	1748
ΣX^2	860666	1184304	696633	638460	1716945	614700
$(\Sigma X)^2/n$	851267	1166429	682282	636531	1652556	611101
Σx^2	9399	17875	14351	1929	64389	3599
ΣXY	61732.0	99817.0	52121.0	49972.0	126790.0	47888.0
$(\Sigma X)(\Sigma Y)/n$	61396.7	98993.5	51716.0	49952.0	125858.1	47895.2
Σxy	335.3	823.5	405.0	20.0	931.9	-7.2
ΣY	163	304	140	140	353	137
ΣY^2	4445.0	8448.0	3932.0	3924.0	9613.0	3759.0
$(\Sigma Y)^2/n$	4428.2	8401.5	3920.0	3920.0	9585.3	3753.8
Σy^2	16.8	46.5	12.0	4.0	27.7	5.2
$(\Sigma xy)^2/\Sigma x^2$	12.0	37.9	11.4	.2	13.5	.01
$\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	4.8	8.6	0.6	3.8	14.2	5.2

Appendix C: Regression of 'days pupa' (Y) on 'weight pupa, mgs.' (X) within treatment, within sex

TABLE 1b

Computation of Sums of Squares and Products for 25° C. Data in Table 2

Class	25°,3.2,F	25°,3.2,M	25°,11.9,F	25°,11.9,M	25°,15.8,F	25°,15.8,M
n	7	9	9	5	11	7
ΣX	2601	3721	3179	1880	3937	2343
ΣX^2	988755	1647211	1162735	712540	1434829	797625
$(\Sigma X)^2/n$	966457	1538427	1122893	706880	1409088	784236
Σx^2	22298	108784	39842	5660	25741	13389
ΣXY	73229.0	113467.0	80599.0	48947.0	106776.0	64164.0
$(\Sigma X)(\Sigma Y)/n$	72828.0	114110.7	80534.7	48880.0	105941.1	63595.7
Σxy	401.0	-643.7	64.3	67.0	834.9	568.3
ΣY	196	276	228	130	296	190
ΣY^2	5502.0	8744.0	5850.0	3402.0	8000.0	5190.0
$(\Sigma Y)^2/n$	5488.0	8464.0	5776.0	3380.0	7965.1	5157.1
Σy^2	14.0	280.0	74.0	22.0	34.9	32.9
$(\Sigma xy)^2/\Sigma x^2$	7.2	3.8	0.1	0.8	27.1	24.1
$\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	6.8	276.2	73.9	21.2	7.8	8.8

Apprendix C: Regression of 'days pupa' (Y) on 'weight pupa, mgs.' (X) within treatment, within sex

TABLE 1c

Computation of Sums of Squares and Products for 30° C. Data in Table 2

Class	30°,3.2,F	30°,3.2,M	30°,11.9,F	30°,11.9,M	30°,15.8,F	30°,15.8, M
n	8	8	8	11	11	2
ΣX	2871	2671	2750	3480	3764	606
ΣX^2	1039935	915791	954092	1107242	1290776	184130
$(\Sigma X)^2/n$	1030330	891780	945313	1100945	1287972	183618
Σx^2	9605	24011	8779	6297	2804	512
ΣXY	69478.0	66022.0	69012.0	87650.0	92433.0	14544.0
$(\Sigma X)(\Sigma Y)/n$	69262.9	65773.4	68750.0	87632.7	92389.1	14544.0
Σxy	215.1	248.6	262.0	17.3	43.9	--
ΣY	193	197	200	277	270	48
ΣY^2	4705.0	4863.0	5052.0	6985.0	6664.0	1152.0
$(\Sigma Y)^2/n$	4656.1	4851.1	5000.0	6975.4	6627.3	1152.0
Σy^2	48.9	11.9	52.0	9.6	36.7	--
$(\Sigma xy)^2/\Sigma x^2$	4.8	2.6	7.8	.1	.7	--
$\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	44.1	9.3	44.2	9.5	36.0	--

Appendix C:

TABLE 2

Regression Data in 18 Classes of Pupa

Class				Sums of squares and products			Errors of estimate	
Temp.	S.D.	Sex	d.f.	$\leq x^2$	$\leq xy$	$\leq y^2$	SS	d.f.
20° C.	3.18	F	5	9399	335.3	16.8	4.8	4
		M	10	17875	823.5	46.5	8.6	9
	11.88	F	4	14351	405.0	12.0	0.6	3
		M	4	1929	20.0	4.0	3.8	3
	15.78	F	12	64389	931.9	27.7	14.2	11
		M	4	3599	-7.2	5.2	5.2	3
25° C.	3.18	F	6	22298	401.0	14.0	6.8	5
		M	8	108784	-643.7	280.0	276.2	7
	11.88	F	8	39842	64.3	74.0	73.9	7
		M	4	5660	67.0	22.0	21.2	3
	15.78	F	10	25741	834.9	34.9	7.8	9
		M	6	13389	568.3	32.9	8.8	5
30° C.	3.18	F	7	9605	215.1	48.9	44.1	6
		M	7	24011	248.6	11.9	9.3	6
	11.88	F	7	8779	262.0	52.0	44.2	6
		M	10	6297	17.3	9.6	9.5	9
	15.78	F	10	2804	43.9	36.7	36.0	9
		M	1	512	--	--	--	--
	Sums		123	379264	4587.2	729.1	575.0	105

Appendix C:

TABLE 3

Analysis of Errors of Estimate from Average Regression Within Groups

Source of variation	d.f.	SS	MS
Deviations from average intra-class regression, $729.1 - \frac{(4587.2)^2}{279264}$	122	673.6	
Deviations from individual intra-class regressions	105	575.0	5.48
Differences among intra-class regressions	17	98.6	5.80

Since the 18 individual regression coefficients do not differ significantly (Table 3), they were pooled to give an average intra-class regression coefficient, b_a, viz.,

$$b_a = \Sigma\Sigma xy / \Sigma\Sigma x^2 = \frac{4587.2}{379264.0} = 0.01209$$

Standard error s_b was calculated from the mean square of deviations from average regression

$$s_b = \sqrt{\frac{673.6/122}{379264}} = \sqrt{\frac{5.52}{379264}}$$

$$\sqrt{0.00001455} = 0.00382$$

To test b_a against zero,

$$t = b_a/s_b = 0.01209/0.00382 = 3.16^{xx}$$

which with 122 d.f. is significant at the 1% level.

Thus the average intra-class regression coefficient was

$$0.012 \pm 0.004$$

or an average increment of 10 mg. in pupal weight was associated with an average increment of approximately 1/8 day in pupal period.

Alternatively, the observed relationship between 'pupal weight' and 'pupal days' can be expressed with reference to the intra-class correlation coefficient

$$r = \frac{\Sigma\Sigma xy}{\sqrt{\Sigma\Sigma x^2 \, \Sigma\Sigma y^2}} = \frac{4587.2}{\sqrt{276,521,382.4}}$$

$$\frac{4587.2}{16629} = .276$$

$$r^2 = (.276)^2 = .076$$

Therefore approximately 8% of the intra-class variation of the variable pupal days was associated with pupal weight.

Appendix D: Analysis of variance for days prepupa

1. Correction factor: $(\leq x)^2/n = \frac{(179.3263)^2}{141} = \underline{\underline{228.0704}}$

2. Total: $\leq x^2 - c = 235.8151 - 228.0704 = \underline{\underline{7.7447}}$

3. Sub-classes: $\frac{(25.9599)^2}{24} + \frac{(22.6387)^2}{21} + \frac{(34.1917)^2}{27} +$

$\frac{(25.0880)^2}{21} + \frac{(40.0717)^2}{27} + \frac{(31.3763)^2}{21} -$

$228.0704 = \underline{\underline{4.0371}}$

4. Within sub-classes: $7.7447 - 4.0371 = \underline{\underline{3.7076}}$

5. Temperature: $\frac{(48.5986)^2}{45} + \frac{(59.2797)^2}{48} + \frac{(71.4480)^2}{48} -$

$228.0704 = \underline{\underline{3.9750}}$

6. Sex: $\frac{(100.2233)^2}{78} + \frac{(79.1030)^2}{63} - 228.0704$

$= \underline{\underline{0.0299}}$

7. Interaction: Temp. x Sex.

$4.0371 - (3.9750 + .0299) = \underline{\underline{0.0322}}$

Analysis of Variance for Days Prepupa

Source of Variance	d.f.	Sums of Squares	Mean Square	F
Temperature	2	3.9750	1.9875	148.8764**
Sex	1	0.0299	0.0299	2.2397
Temp. x Sex	2	0.0322	0.0161	1.2059
Within Sub-classes	135	1.8029	0.01335	
Total	140	7.7447		

Appendix 3: Analysis of variance for some results

1. Germination Energy: $\frac{(\quad)^2}{\quad}$ $\frac{(\quad)^2}{\quad}$ $\frac{(\quad)^2}{\quad}$

Appendix E: Analysis of variance for days pupa

1. Correction Factor: $(\leq x)^2/n = (200.3808^2/141 = \underline{284.7692}$

2. Total: $\leq x^2 - c = 285.0422 - 284.7692 = \underline{0.2730}$

3. Sub-classes: $\frac{(34.4634)^2}{24} + \frac{(30.2656)^2}{21} + \frac{(38.4525)^2}{27} +$

$$\frac{(30.4213)^2}{21} + \frac{(37.4823)^2}{27} + \frac{(29.2957)^2}{21} -$$

$$284.7692 = \underline{0.0736}$$

4. Within sub-classes: $.2730 - .0736 = \underline{0.1994}$

5. Temperature: $\frac{(64.7290)^2}{45} + \frac{(68.8738)^2}{48} + \frac{(66.7780)^2}{48}$

$$284.7692 = \underline{0.0655}$$

6. Sex: $\frac{(110.3982)^2}{78} + \frac{(89.9826)^2}{63} - 284.7692$

$$= \underline{0.0059}$$

7. Interactions: Temp. x Sex.

$$.0736 - (.0655 + .0059) = \underline{0.0022}$$

Analysis of Variance for Days Pupa

Source of Variance	d.f.	Sums of Squares	Mean Square	F
Temperature	2	.0655	.0328	65.6**
Sex	1	.0059	.0059	11.8**
Temp. x Sex	2	.0022	.0011	2.2
Within Sub-classes	135	.0736	.0005	
Total	140	.1472		

Appendix 13. Analysis of variance for dress dyes

Source	DF			
Total	179	.7174		
Cabin mate-classes	136	.4924	.0036	
Days	2	.0030	.0015	1.8
Error	178	.4894		

Appendix 14. Analysis of variance for dress dyes

Source of Variance	D.F.	Sum of Squares	Mean Square	F
Temperature	2	.3893	.1946	[illegible]
Days	2	.0030	.0015	1.8
Error	176	.4894	.0028	
Total	180	.7174		